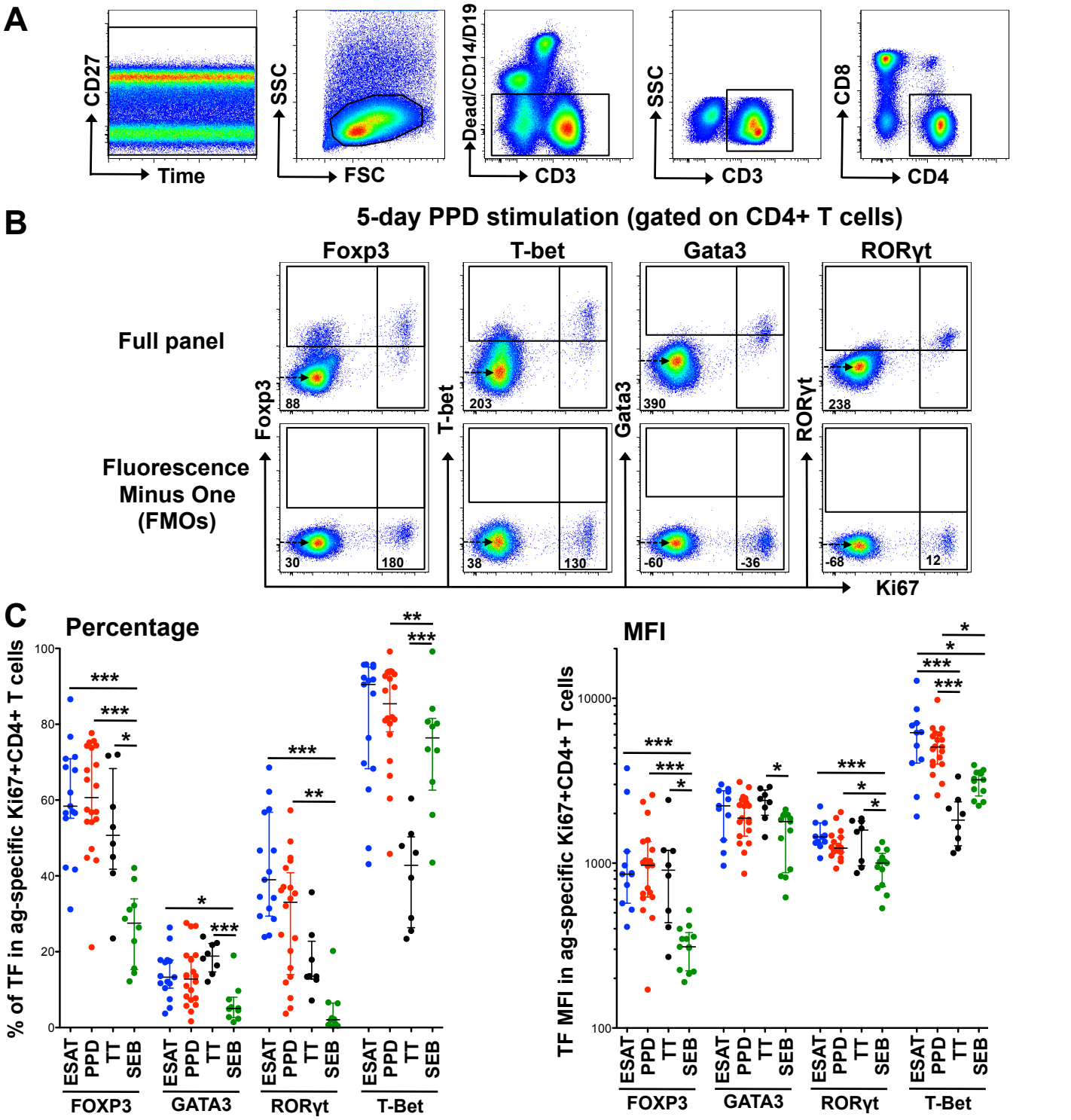
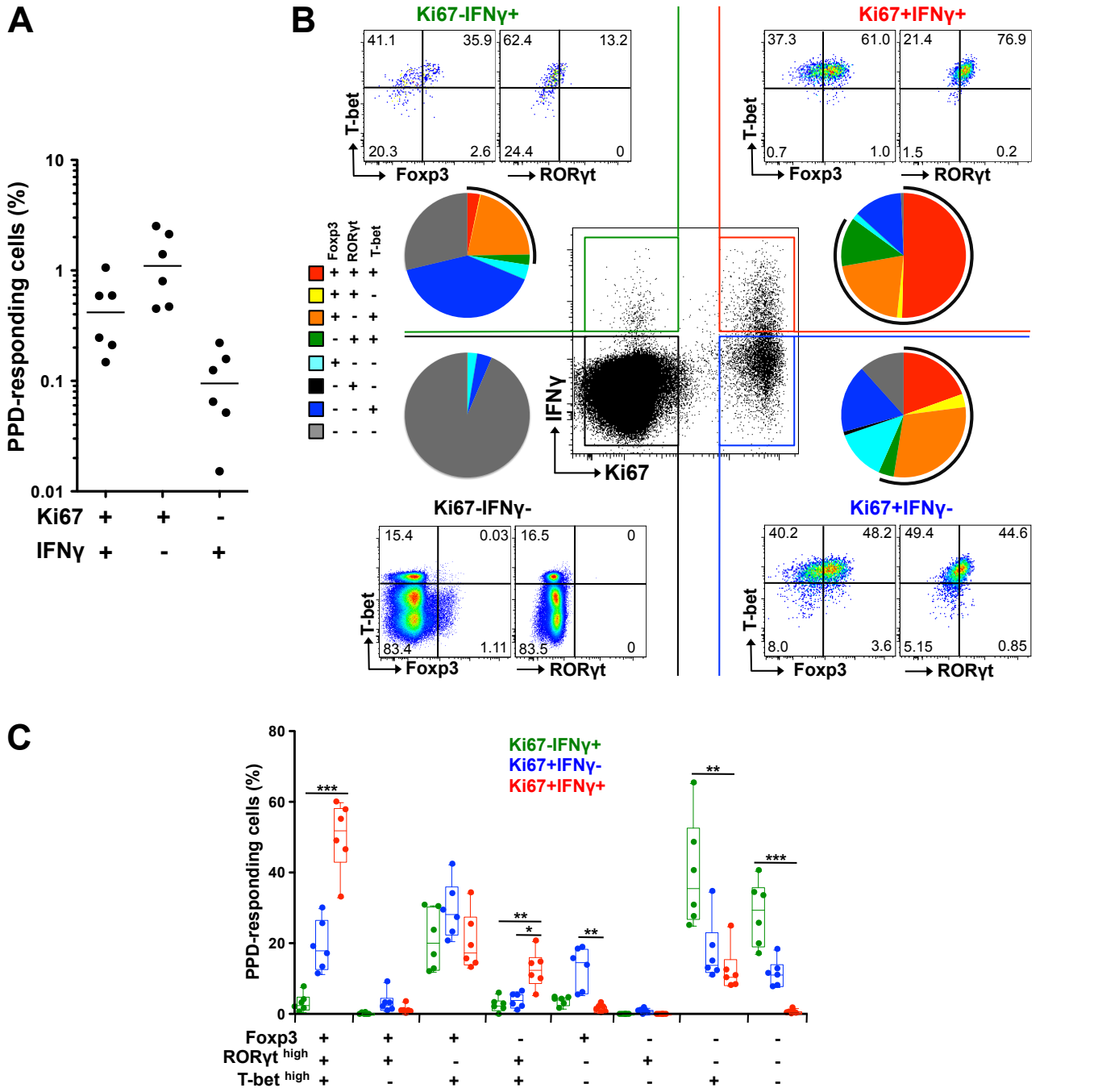
Supplemental Figure S1



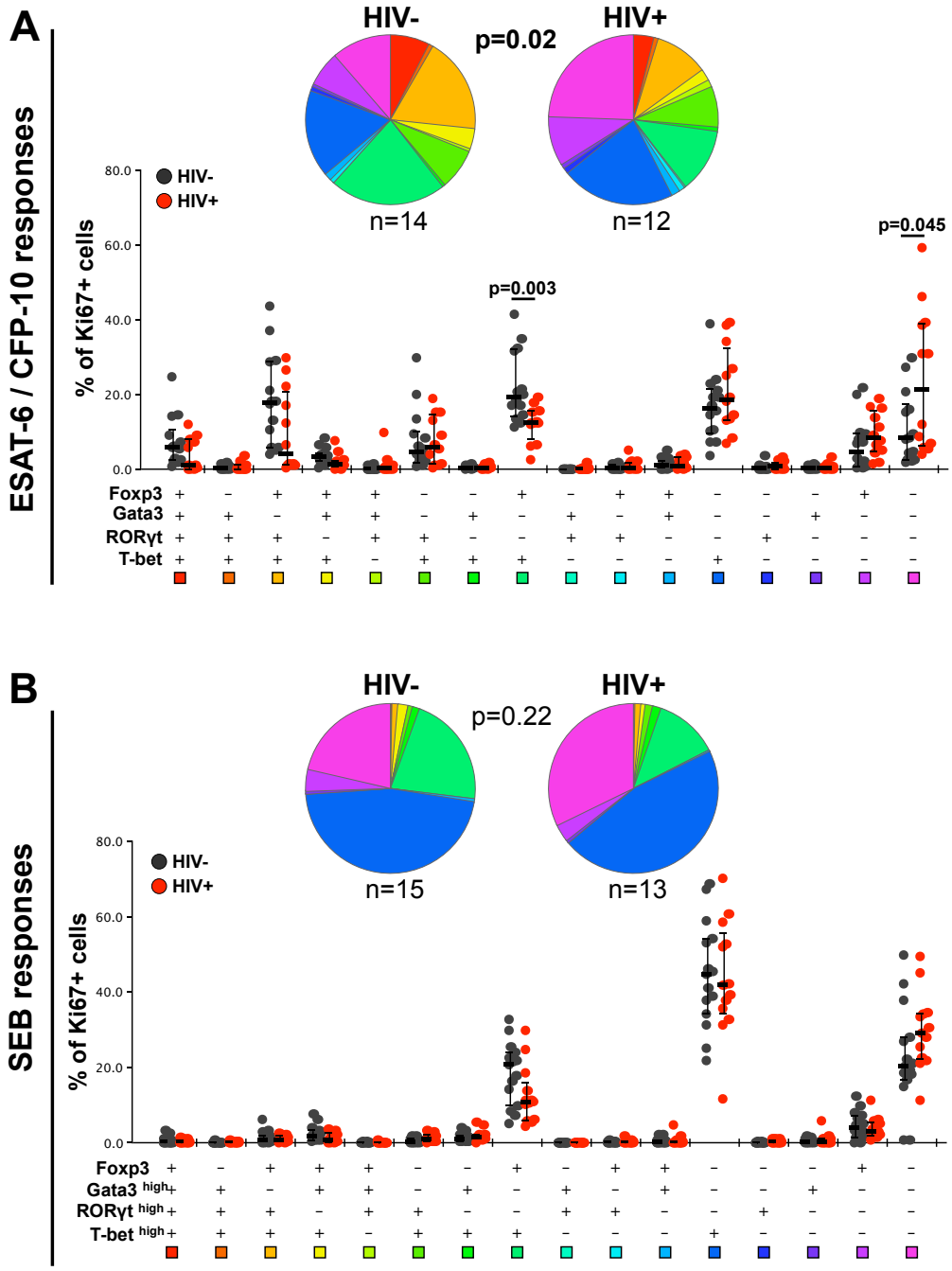
Supplemental Figure S1: **Gating strategies. (A)** Gating strategy for CD4+ T cells. **(B)** Gating strategy for the lineage-defining transcription factors in antigen-specific CD4+ T cells after a 5-day PPD stimulation. Representative dot plots show the expression of each transcription factor (Foxp3, T-bet, Gata3 and RORγt) and their corresponding FMO controls in PPD-specific Ki67+CD4+ T cells in one HIV-uninfected individual. The numbers correspond to the median fluorescence intensity of each transcription factor in Ki67- or Ki67+CD4+ T cells. **(C)** Comparison of the expression level of lineage defining transcription factors in antigen-specific CD4+ T cells using percentage or MFI. Proportion of TF positive cells within ESAT6/CFP10-, PPD-, Tetanus Toxoid (TT)- and SEB-specific CD4+ T cells expressed as a percentage or MFI of Ki67+CD4+ T cells. Each dot represents an individual. Median and interquartile ranges are shown. Statistical comparisons were performed using an ANOVA test. Of note, While RORγt and Gata3 show a unimodal distribution, these populations are heterogeneous (i.e. cells expressing high or low levels of RORγt and Gata3 were significantly distinct based onT-bet and Foxp3 expression levels in a 5-day proliferation assay), lending credence to the division of these populations based on their MFI for Boolean gating analyses.

Supplemental Figure S2



Supplemental Figure S2: **Transcription factor expression profile of PPD-specific cells depending on their IFN-γ secretion and/or proliferation potential. (A)** Frequency of CD4+ T cells expressing IFN-γ and/or Ki67 in response to a 5 day PPD stimulation (n=6). **(B)** Representative example of TF expression profile (T-bet, RORγt and Foxp3) in PPD-responding CD4+ T cells depending to their ability to secrete IFN-γ and/or proliferate (Ki67+). The pies show the median proportion of each possible combination of TF expression in Mtb-specific CD4+ T cells. Each slice of the pie corresponds to a distinct combination of TF. The black arc illustrates the proportion of PPD-specific CD4+ T cells expressing multiple (≥ 2) transcription factors. **(C)** Comparison of the proportion of PPD-responding cells expressing 3, 2, 1 or none of the TF measured between IFN-γ+KI67- (green), IFN-γ-KI67+ (blue) and IFN-γ+KI67+ (red) CD4+ T cells. Bars represent median and interquartile ranges. Statistical comparisons were performed using a Student's t-test. *<0.05, **<0.01, ***<0.001.

Supplemental Figure S3



Supplemental Figure S3: Comparison of TF expression profiles in ESAT-6/CFP-10- (A) and SEB (B) responsive CD4+ T cells in HIV-infected and HIV-uninfected individuals. Pie charts show the median proportion of each possible TF combination within antigen-responsive Ki67+CD4+ T cells in HIV-infected and HIV-uninfected individuals. Statistical comparisons were performed using the pie statistic tool integrated in the Spice software. Graphs show the proportion of cells expressing each possible TF combination in antigen-responsive CD4+ T cells using a Boolean gating strategy. HIV-infected individuals are depicted with red dots and HIV-uninfected individuals with black dots. Bars represent median and interquartile ranges. Statistical comparisons were performed using the Student's t-test.